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Habitual tea consumption protects against the inhibitory effects of tea on iron absorption in rats

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Abstract

The hypothesis of this study was that tea inhibits iron absorption in animals unaccustomed to tea consumption but not in animals habituated to high intakes of tea. Three diets were prepared: 1) tannin-free diet; 2) 5% tea solids (tea) diet; and 3) 5% tea solids + 6% gelatin (T/G). Gelatin was chosen as a proxy for salivary proline–rich proteins (PRP). Five groups of rats were assigned to the following treatments: 1) control (tannin-free diet throughout), 2) short-term tea (tannin-free except tea diet on day 8, 3) long-term tea (tea diet throughout), 4) short-term T/G (tannin-free except tea with gelatin diet on day 8, and 5) long-term T/G (tea with gelatin diet throughout). After 10 days of dietary treatment, iron absorption, measured using ^{59}Fe, was 41%, 7%, 25%, 7%, and 21% for treatments 1, 2, 3, 4, and 5, respectively. Rats in groups 3 and 5 showed higher PRP than control. We conclude that rats possess adaptive mechanisms for partially overcoming the inhibitory effects of tannins on iron absorption possibly by increasing secretion of PRP in saliva.

Keywords: Iron absorption; Bioavailability; Tea; Tannin; Salivary protein; Proline rich protein

1. Introduction

Iron absorption is consistently depressed by tea in both rats [1,2] and humans [3–8] when measured in short-term studies, presumably by forming iron complexes in the gastrointestinal tract. Epidemiological studies, on the other hand, do not seem to support this observation. An epidemiological study with National Health and Nutrition Examination Survey

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(NHANES II) participants [9] showed a negative correlation between tea intake and iron deficiency. These conflicting results may be due to differences in the duration of exposure to tannins, as it is known that prolonged tannin ingestion triggers defensive mechanisms against tannin toxicity.

Tannins are toxic to insects and may play a role of deterring insect attacks on plants [10,11]. Several reports suggest that some insects have a defensive mechanism to protect them against tannin toxicity. A similar physiological adaptation may also exist in mammals. High-tannin diets produced a dramatic hypertrophic effect on parotid salivary glands and an associated induction of proline-rich salivary proteins (PRP) in rats [12,13] and mice [14]. Because PRP exhibit a very high affinity for tannins [15], tannins may bind selectively to PRP even in the presence of a large excess of other proteins with marginal or average affinities for tannins. Thus PRP synthesized by the parotid glands in response to high dietary tannin intake may function as tannin-binding agents and defend the animal against deleterious effects of tannins. A similar defensive response in higher animals, including humans, is expected, since as PRP are major components of saliva of humans as well as other animals [16]. Lu and Bennick [17] reported that the protein in human parotid secretions is more than 70 % PRP and most of the condensed tannin-PRP complexes remained insoluble under conditions similar to those in the stomach and small intestine, supporting the hypothesis that PRP act as a defense against tannins.

The objective of this study was to test the hypothesis that tea inhibits iron absorption in animals unaccustomed to tea consumption but not in animals habituated to high intakes of tea. The rationale for this hypothesis is that animals adapt to habitual tannin ingestion by secreting PRP in saliva, which complexes with dietary tannins, thereby preventing them from binding iron. Effects of dietary PRP were also tested using diets supplemented with calf-skin gelatin. Hagerman and Butler [15] showed that calf-skin gelatin (18 mol % proline and hydroxyproline) and rat parotid PRP (43 mol % proline) were the most effective at binding tannins of all naturally occurring polymers tested.

2. Methods and materials

2.1. Diet formulation

Diets were formulated to meet recommended nutrient levels for rats (AIN-76A diet, AIN 1980) (Table 1). All diets contained 20% protein provided as either casein or gelatin (Flaked-50 Bloom, Type A, Sigma Chemical Co., St. Louis, MO). Tryptophan, phenylalanine, and tyrosine, limiting amino acids in gelatin, were added to make the amino acid score of gelatin-containing diets similar to that of the other diets. In the tea-containing diets, tea solids were substituted for cellulose and sucrose.

Tea was brewed in 1 L batches by adding 40 g of black tea (U.S. Tea Association, Black Tea Research Blend, Thomas J. Lipton Co., Englewood Cliffs, NJ) to 1 L boiling distilled, deionized water, and allowing it to steep at room temperature for 30 min. The brewed tea was filtered by gravity using Whatman no. 1 quality filter paper (Whatman Ltd., England). Tannin concentration in the brewed tea was 210.6 μg/mL catechin equivalents and 368.9 μg/mL

Table 1
Composition of experimental diets (by formulation)

Ingredient	Experimental diet				
	Control	Tea	T/G	½ Tea	½ T/G
Protein	(g/kg)				
Casein*	200	200	140	200	166
Gelatin†	—	—	60	—	34
Fat					
Corn oil	50	50	50	50	50
Carbohydrate					
Starch	150	150	150	150	150
Sucrose	500	480	477	490	487
Cellulose	50	20	20	35	33
Choline	2	2	2	2	2
AIN-76A vitamin mix	10	10	10	10	10
AIN-76 mineral mix	35	35	35	35	35
DL-methionine	3	3	3	3	3
Amino acids					
L-phenylalanine	—	—	0.21	—	0.12
L-tyrosine	—	—	3.29	—	1.82
DL-tryptophane	—	—	0.10	—	0.06
Tea solids	—	50	50	25	25

* Casein, 95% protein, dry basis.
† Gelatin, Flaked-50 Bloom, Type A (ICN Biomedicals Inc., Cleveland, OH).
T/G = tea+gelatin.

tannic acid equivalents as determined by measuring the concentration of iron-binding phenolic groups [18]. To prepare the tea-containing diets, 5 L of brewed tea was mixed with 1 kg of the dry ingredients, and the mixture was freeze-dried and ground (sample mill, Cemotoc, 1090, Tecator, Hoglana, Sweden). Diets were analyzed for protein content [19], tannin concentration [18], and total iron concentration [20] (Table 2).

The tannin content of the tea and T/G diets was 0.9% and 1% tannic acid equivalents, respectively. Diets containing 0.5% tannin (1/2 tea or 1/2 T/G) were prepared by diluting tea or T/G diets with the standard AIN-76A diet. The diets were stored at 4°C until used.

2.2. Rat housing and care

The study protocol was approved by an Institutional Animal Care and Use Committee at Cornell University. Weanling male Sprague-Dawley rats (Camm Research Institute, Wayne,

Table 2
Analyzed composition of experimental diets

	Control	Tea	T/G	½ Tea	½ T/G
Protein (g/kg diet)	182.6 ± 1.9	196.2 ± 5.4	2040 ± 7.7	189.4 ± 2.0	193.9 ± 2.0
Tannic acid equivalents (g/kg diet)	0.3 ± 0.0	8.9 ± 0.5	9.9 ± 0.7	5.0 ± 0.0	5.0 ± 0.0
Total iron (mg/kg diet)	35.9 ± 1.3	30.7 ± 3.1	33.2 ± 2.3	34.3 ± 1.5	34.6 ± 1.0

Values are mean ± SD for each diet group, $n = 3$.

Table 3
Experimental design

Treatment	Adjustment period (day −4 to 0)	Experimental period					
		days 1–7	day 8	day 9–10	Overnight	day 11	days 11–24
		Diet					
Control		Control	Control	Control		^{59}Fe-control	CD
ST	Commercial diet (CD)	CD	½ Tea	CD	Fasted for 14 hours	^{59}Fe-Tea	CD
LT		Tea	½ Tea	½ Tea		^{59}Fe-Tea	½ Tea
ST/G		CD	½ T/G	CD		^{59}Fe-T/G	CD
LT/G		T/G	½ T/G	½ T/G		^{59}Fe-T/G	½ T/G

ST = short-term tea diet ingestion; LT = long-term tea diet ingestion; ST/G = short-term tea + gelatin diet ingestion; LT/G = long-term tea + gelatin diet ingestion; CD = commercial diet (Prolab 1000, Agway, Ithaca, NY).

NJ) were purchased. They were housed individually in suspended stainless steel cages with wire mesh bottoms in a humidity- and temperature-controlled room with a reverse 12-hour light:dark cycle. The rats had free access to distilled water and a commercial rat diet (Prolab 1000, Agway, Ithaca, NY) for 5 days prior to the start of the study. On study day 0, blood samples were obtained from the tail and hemoglobin concentrations were determined by a cyanmethemoglobin method (Sigma kit 525-A, Sigma Chemical, St. Louis, MO). The rats were then allocated to five groups of six rats each so that each group had similar mean hemoglobin concentration and body weight.

Groups were allocated randomly to five different treatments: control, short-term tea (ST), long-term tea (LT), short-term T/G (ST/G), and long-term T/G (LT/G). The control group was fed the standard AIN-76A diet for the entire 24 days of the experimental period. Rats in short-term treatment groups were fed the standard diet except for study day 8, when they were fed either the 1/2 tea or 1/2 T/G diet. Rats in the long-term treatment groups were fed tea-containing diets for 10 days (original tea-containing diet for study days 1–7 and 1/2 tea or 1/2 T/G diets for study days 8–10) prior to dosing with an ^{59}Fe-labeled test meal. After dosing, rats were fed their respective 1/2 tea or 1/2 T/G diets for the rest of the experiment. The experimental protocol is described in Table 3. Body weights and feed intakes were monitored for 10 days until the ^{59}Fe-labeled test meals were given.

2.3. Assessment of iron absorption

After the experimental diets were fed to the rats for 10 days, the rats were deprived of food for 14 hours. Then the rats were offered a 2-g meal of their respective diet labeled extrinsically with $^{59}FeCl_3$, (18.5 kBq in 0.1 mL of 0.01 mol/L HCl, specific activities of 598.4 kBq/g total Fe) (Dupont/New England Nuclear, North Billerica, MA). Meals were consumed within 10 min. Three hours after ingesting the meal, rats were counted individ-

ually in a custom made, well-type, whole-body gamma counter at a window setting of 0.930–1.500 meV for 50 seconds to determine the amount of the isotope ingested. It was assumed that no isotope was excreted before the first whole-body count. Subsequently, the rats were assayed at 24-hour intervals for 1 week and at 48-hour intervals for 1 week subsequently to monitor ^{59}Fe retention. Radioactivity from a ^{59}Fe standard obtained during each counting session was used to correct for radioactivity decay.

The rats were given free access to their respective unlabeled diets and to distilled water for these 2 weeks. Groups receiving short-term exposure to tea (ST) or tea + gelatin (ST/G) diets were given a commercial rat diet (Prolab 1000, Agway, Ithaca, NY) for this period. Absorption of ^{59}Fe was estimated from ^{59}Fe retention data determined by whole-body counting [21,22].

2.4. Biochemical analyses

At the end of the study, all rats were weighed and blood samples were obtained from the tail. All rats were killed by over exposure to CO_2 and parotid glands and liver were removed. Parotid glands were rapidly excised, washed in isotonic saline solution, dissected free from adhering tissue, weighed and stored at −20°C. Frozen parotid glands from rats in each group (six rats in control group, four rats in LT, and five rats in LT/G) were pooled together, subsequently thawed and the PRP content of the glands was determined by the method of Mehansho et al. [12]. Nonheme iron in liver was determined by the method of Rhee and Ziprin [23] to assess the iron status of each rat.

2.5. Statistical methods

Analyisis of variance and Fisher's least significant difference method of multiple comparisons were used to detect possible significant differences among means using Minitab software (Minitab, State College, PA). The confidence interval for statistical significance was 95 % ($P < 0.05$).

3. Results

Initial and final mean body weights and hemoglobin concentrations for each treatment group are presented in Table 4. Long-term ingestion of tea and tea + gelatin diets significantly reduced weight gain. There were no differences in final hemoglobin concentration among treatment groups. Initially, the rats in LT or LT/G groups were fed diets that contained 0.9% or 1.0% tannin, respectively. This level of tannin was poorly tolerated by the young rats. One animal in the LT group died on day 5 and another on day 6. One rat in the LT/G group died on day 7. Consequently, the tannin contents of the diets were lowered to 0.5%, and this level of tannin was fed from day 8 to day 24. Intakes of treatment diets from the beginning of the experimental period until the day before dosing with ^{59}Fe are shown in Fig. 1.

Daily feed consumption by rats fed the standard diet was greater than that of rats fed diets

Table 4
Initial and final body weight and hemoglobin concentration for each treatment group

Group	Initial			Final		
	No. of animals	Body weight (g)	Hb concentration (g/L)	No. of animals	Body weight (g)	Hb concentration (g/L)
Control	6	67.0 ± 10.1[a]	121.5 ± 6.2[a]	6	246.5 ± 30.9[a]	138.4 ± 15.0[a]
ST	6	67.2 ± 8.8[a]	121.6 ± 6.6[a]	6	245.6 ± 12.9[a]	126.3 ± 23.2[a]
LT	6	67.2 ± 7.5[a]	121.7 ± 9.6[a]	4	167.5 ± 2.5[b]	143.3 ± 2.3[a]
ST/G	6	67.2 ± 6.6[a]	121.4 ± 8.9[a]	6	239.0 ± 14.8[a]	133.8 ± 15.9[a]
LT/G	6	66.8 ± 8.6[a]	122.5 ± 5.4[a]	5	183.2 ± 60.3[b]	130.4 ± 9.2[a]

Values are mean ± SD for each diet group. Means in each column with different superscript letters are significantly ($P < 0.05$) different.

ST = short-term tea diet ingestion; LT = long-term tea diet ingestion; ST/G = short-term tea + gelatin diet ingestion; LT/G = long-term tea + gelatin diet ingestion.

containing tea. Including gelatin in the diet did not ameliorate the general depressant effect of tannin on feed intake. Feed efficiency was calculated by dividing weight change by feed intake (Fig. 2). Feed efficiency for tea and T/G groups was negative initially but eventually recovered to the level of the control group.

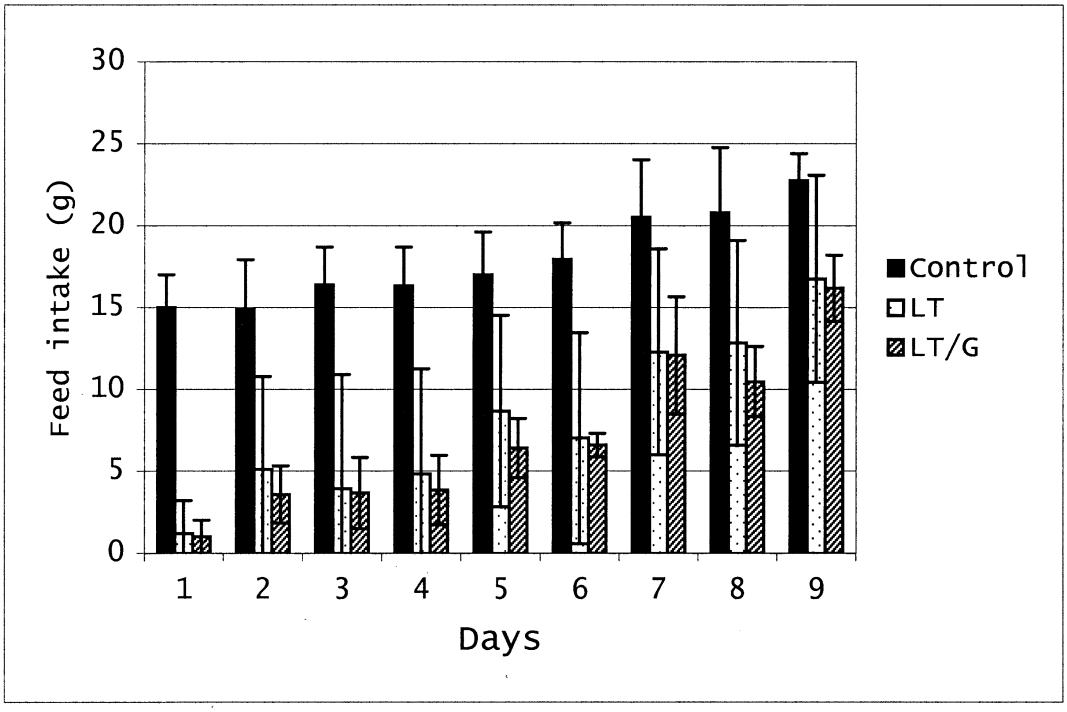

Fig. 1. Intakes of control (no tea), LT (long-term tea) and LT/G (long-term tea + gelatin) diets over the course of the 10-day experiment before dosing with ^{59}Fe. Values are means ± SD (See Table 3 for number of animals).

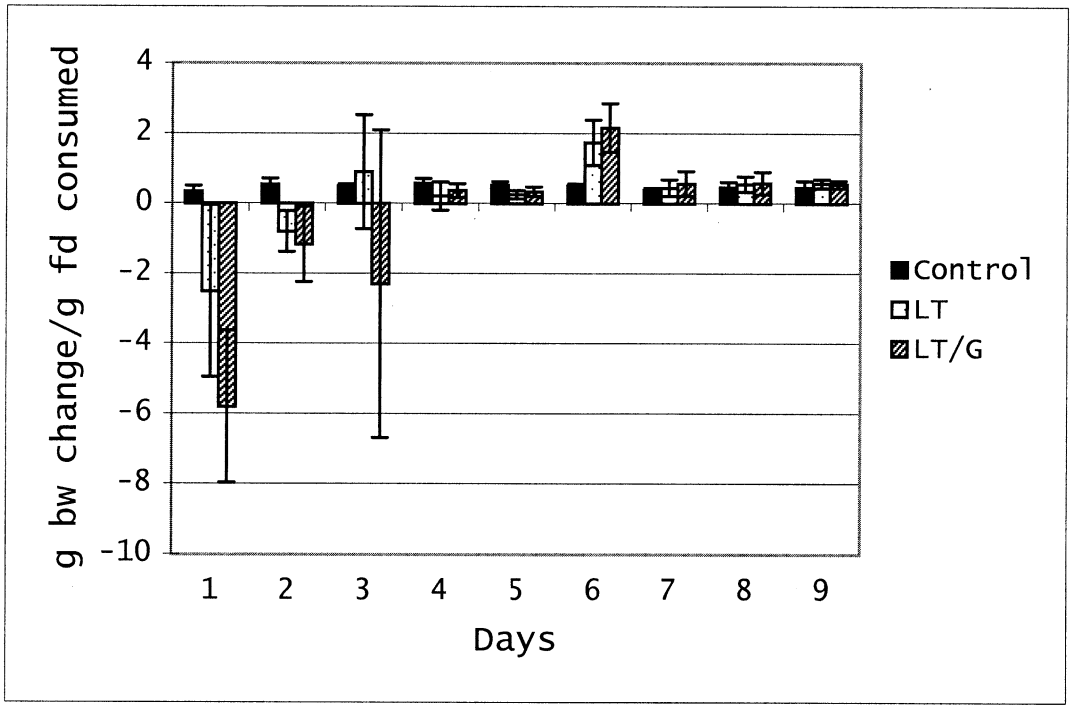

Fig. 2. Feed efficiency in rats fed control (no tea), LT (long-term tea) and LT/G (long-term tea + gelatin) diets over the course of the 10-day experiment before dosing with ^{59}Fe. Feed efficiency was calculated by dividing weight change by feed intake. Values are means ± SD (See Table 3 for number of animals).

Iron absorption and nonheme iron contents in liver of rats in each treatment group are presented in Table 5. Iron absorption was significantly reduced by the tea or T/G diets for both long-term and short-term ingestion. However, animals accustomed to tea in the diet (LT or LT/G) showed significantly greater absorption of ^{59}Fe compared to animals unaccustomed

Table 5
Absorption of ^{59}Fe calculated from whole-body retention data in rats fed meals extrinsically labeled with ^{59}Fe and nonheme iron contents of liver samples

Group	No. of animals	Iron absorption (%)	Liver nonheme iron μg tissue
Control	6	41.44 ± 10.88[a]	44.73 ± 3.41[a]
ST	6	7.48 ± 2.26[b]	43.47 ± 3.47[a]
LT	4	25.06 ± 7.41[c]	58.45 ± 5.22[a]
ST/G	6	6.82 ± 1.58[b]	46.64 ± 3.57[a]
LT/G	5	20.75 ± 4.53[c]	58.82 ± 6.91[a]

Values are mean ± SD for each diet group. Means in each column not sharing a common superscript letter are significantly ($P < 0.05$) different.

ST = short-term tea diet ingestion; LT = long-term tea diet ingestion; ST/G = short-term tea + gelatin diet ingestion; LT/G = long-term tea + gelatin diet ingestion.

Table 6
Weights of parotid salivary glands and PRP production

Group	No. of animals	Gland weight (mg/g bw)*	PRP production (μg bw)†
Control	6	1.02 ± 0.08[a]	4.18
LT	4	1.53 ± 0.50[b]	5.08
LT/G	5	1.49 ± 0.24[b]	4.58

Values are mean ± SD for each diet group.
* Mean with different superscript letter is significantly ($P < 0.05$) different.
† Values are from pooled gland samples of animals in the same group.
LT = long-term tea diet ingestion; LT/G, long-term tea + gelatin diet ingestion; bw = body weight.

to tea (ST or ST/G). Iron status of the rats was determined by liver nonheme iron concentraton at the conclusion of the experiment (Table 5). Iron status did not differ significantly among the groups.

Significant hypertrophy of parotid glands was observed in the LT and LT/G groups (Table 6). The PRP production was not significantly different among control, LT, and LT/G groups, although statistical analysis was not conducted because protein production was assayed from the pooled glands of each group.

4. Discussion

Tannin concentrations were measured with an assay based on iron binding by phenolic groups [19]. Although the vanillin assay is most widely used for quantitative measurement of condensed tannin (proanthocyanidins), it does not measure tannins that do not contain resorcinol groups such as gallic acid and tannic acid. Gallic and tannic acids are known to bind iron. Therefore, the method to determine iron-binding phenolic groups is more appropriate for the purposes of the present study than other commonly used methods. Brune et al. [5] used this method to show a relationship between the content of iron-binding galloyl groups in foods and the degree of inhibition of iron absorption. According to these authors, among iron-binding phenolic compounds in food, the galloyl group is the structure mainly responsible for inhibition of iron absorption.

Tea ingestion significantly reduced weight gain and feed efficiency in agreement with other reports [24,25]. However, feed efficiency in tea-fed animals gradually recovered to levels of the control group as the animals adapted to the tea diets. Given that tea-containing diets were switched to diluted diets on day 8 and feed efficiency recovered to positive values by day 3 of tea and day 4 of tea + gelatin diets, switching diets was not the only reason for the improvement in feed efficiency. Reduction of iron absorption by tea-containing meals was much less in animals fed tea throughout the study compared with animals fed tea for 1 day only. Groups ST and ST/G were fed a tea or T/G diet on day 8, 3 days before dosing with ^{59}Fe, because rats produce PRP in response to tannin within 2–3 days of tannin ingestion [13]. To compare the defensive response against tannins between short-term and long-term ingestion of tea, we treated animals for either 1 day on day 8 (ST and ST/G) or for the entire

experimental period (LT and LT/G). The partial improvement of ^{59}Fe absorption seemed to result from the incomplete adaptation of the rats. The iron status of animals as assessed by liver iron stores was not different among the treatment groups. Therefore, it is not the iron status of animals but the adaptation that caused the improved iron absorption in the groups fed tea-containing diets throughout the study (LT and LT/G) compared to the groups fed tea-containing diets for only 1 day (ST and ST/G). This is supported by the observed hypertrophy of parotid salivary glands and the production of PRP by the rats in the long-term tea-containing diet groups (Table 6). Mehansho et al. [12–14] reported that high-tannin diets fed to rats or mice resulted in enlarged parotid salivary glands and increased synthesis of proline-rich salivary proteins. The maximal increases in average tissue weight and PRP production of our study were about 50% and 20%, respectively. These values are considerably less than the 3-fold increase in tissue weight reported for rats fed high-tannin sorghum [12] and the 10-fold increase in PRP production of rats fed high-tannin sorghum [13]. The smaller response observed in the present study may reflect differences in either the amount or chemical structure of the tannins. The dietary concentration of tannin was 0.5% in our study and about 7% in studies [12,13] with high-tannin sorghum. Apparently, with a similar level of bean tannin (0.5%), a similar magnitude of hypertrophy was observed [26]. For fast-growing young animals, dramatic weight loss or limited nutrient intake during the early stage of their development could be too stressful to recover their full physiological functions even after they consumed an adequate amount of nutrients. Growing rats in the present study showed less hypertrophy of the salivary glands compared to mature rats in the study by House and Van Campen [26]. Unpublished observations from the follow-up experiments by the authors conducted with mature rats (mean body weight 300 g) also showed about a 2.4-fold hypertrophy (tissue weight of 0.88g for the control group versus 2.13 g for the tannin group). In an epidemiological study, Merhav et al. [27] showed that tea drinking was associated with lower hemoglobin concentrations in infants. On the other hand, Mehta et al. [4] reported that coffee and tea drinking were negatively associated with anemia for NHANES II participants. These studies also suggest that effects of tea may be different for different age groups. Therefore, tea could be more effective at reducing iron absorption in younger animals or children than adults.

Gelatin is rich in proline and has a high affinity for tannins [15]. Because of its high affinity for tannins, gelatin might be expected to counteract adverse effects of tea by binding to tannins, thereby rendering them unavailable for binding to other proteins and chemical species such as iron. However, gelatin did not show protective effects when it was added to the dried diets before the incorporation of the tea infusion. In contrast, inhibition of growth rates of young rats by high-tannin sorghum was overcome by adding gelatin to the diet [13]. Differences between tea tannins and sorghum tannin or different levels of gelatin used (6% in our study versus 8% in that of Mehansho et al.) may have caused the different results. Tea is normally consumed as a liquid, and we fed the tea in dry form. The sequence of gelatin addition could be more important for tea tannin than for sorghum tannin. When added to liquid tea and then mixed with diet, gelatin showed defensive effects against tea tannin inasmuch as there would be greater opportunity for the proteins and tannins to interact (unpublished data, H.S. Kim and D.D. Miller).

The overall effect of tea on iron absorption was dependent on the duration of tea ingestion.

These findings are consistent with data from studies with human subjects [1,7,8] or rats in single meal absorption studies [5,6].

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